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Determination of nutritive value of forages in south Texas using an *in vitro* gas production technique

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Abstract

The objectives of this study were to use the *in vitro* gas production (IVGP) technique to evaluate the pattern and parameters of anaerobic fermentation of forages from south Texas pastures throughout the year to (i) obtain empirical relationships between the IVGP technique fermentation parameters and chemical composition of the forages and (ii) develop equations to compute total digestible nutrients (TDN). During four consecutive years (2006–2009), forage samples were collected monthly (n = 39) at the King Ranch, TX, and chemical analyses and IVGP were obtained. For 2006, 2007, 2008 and 2009, the average lag times, h, were 6·47 ± 0·54, 7·75 ± 0·65, 7·49 ± 2·01 and 5·44 ± 1·46, and the average ratio of millilitre of gas per milligram of dry matter was 0·41 ± 0·11, 0·34 ± 0·09, 0·34 ± 0·07 and 0·26 ± 0·10 respectively. There was a moderate negative correlation (r = −0·53) between lignin and neutral detergent fibre (NDF) and a moderate positive correlation (r = 0·58) between crude protein and NDF digestibility. The predicted fractional passage rate (kp) by the large ruminant nutrition system model using the level 2 solution was on average 0·0366 h^{-1}. The average computed TDN assuming a kp of 0·04 h^{-1} was 55·9%. We concluded the IVGP technique may be used to predict TDN values of warm-season forages.

Keywords: beef cattle, *in vitro*, modelling, nutrition, prediction, simulation

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Introduction

In a cow/calf system, forage is the major source of energy and protein for the animals. Increases in the duration of grazing period and decreases in the amount of supplementation for beef cows are alternatives to decrease production costs because feed and hay are the major costs in cattle production (Becker, 2008). The main challenge in producing cattle under grazing is to maintain forage nutritive value and dry matter (DM) mass available throughout the year. Therefore, reliable and more rapid forage analyses are needed to accurately determine the availability of energy and nutrients of the forage.

Allen and Segarra (2001) reported that forage quality is best described as the degree to which forage meets the nutritional requirements of a specific kind and class of animal. According to Adesogan (2005), typical techniques (e.g. digestibility trials and *in situ* incubation) to evaluate feeds require expensive facilities and large amounts of time and labour. The *in vitro* gas production (IVGP) technique was developed to predict fermentation of ruminant feedstuffs (Rymer *et al.*, 2005). The IVGP technique has been used to evaluate forages because the fermentation kinetics allow for an evaluation of distinct phases of gas production; therefore, the soluble and insoluble fractions of the forage can be evaluated separately (Makkar, 2004). According to Starks *et al.* (2007), by knowing the *in vitro* dry matter digestibility of feedstuffs, producers can make decisions about fertilizer applications, stocking rate, and supplementary feed management. Therefore, the combination of chemical analyses and the IVGP technique might yield reliable measurements of rates of fermentation of fibre that can be used to determine energy availability of feeds for ruminants.

The objectives of this study were (i) to use the IVGP technique to study the pattern of *in vitro* fermentation

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parameters of forages obtained from pastures throughout the year in south Texas during four consecutive years (2006, 2007, 2008 and 2009); (ii) to obtain empirical relationships between the IVGP technique fermentation parameters and chemical composition of the forages; and (iii) to develop equations to compute total digestible nutrients (TDN).

Materials and methods

Forage collection

Forage grass samples {most dominant species were kleberg bluestem [*Dichanthium annulatum* (Forssk.) Stapf] and coastal bermudagrass (*Cynodon dactylon* L. Pers)} (n = 39) were collected during four consecutive years (2006, 2007, 2008 and 2009) for complete chemical analysis. Forage grass samples were obtained from one of three pastures in which Santa Gertrudis (n = 144) cows were grazing at the King Ranch, Kingsville, TX (27°31′N; 97°55′W), every month, to represent the forage that cows were grazing during that period. Two of the pastures are nearly monocultures of coastal bermudagrass, and the other one is a mix of kleberg bluestem and coastal bermudagrass. Twelve subsample areas were randomly selected for forage collection, and about 1 kg of DM was collected during every collection; the subsamples were collected about 150 m apart. The average size of the pastures was 225 ha (0·70 forage, 0·30 brush canopy). The soil texture of this area varies from clay to sandy loams (Natural Resources Conservation Service, 2010). The vegetation type is predominantly grassland or savannah with other species such as mesquite, cacti and acacias (Gould, 1975).

Warm-season perennial grass samples were hand-plucked at different locations of the pastures that animals were grazing and were to be similar to the diet that cows were consuming. In a previous study, hand-plucked samples and oesophageal samples collected from fistulated steers were found to have similar chemical analyses and digestibility values and with no saliva contamination on the sample (Wallace *et al.*, 1972). De Vries (1995) concluded that hand plucking forage samples is practical, is simple, and can be used to predict diet quality of free-ranging animals with no difference from samples collected from oesophageally cannulated animals.

Forage samples were bagged and, immediately after collection, they were put in a freezer at −20°C. Frozen samples were sent to the Ruminant Nutrition Laboratory at Texas A&M University, College Station, TX, and upon arrival, samples were dried at 65°C in an oven (Lindberg/Bluem Model: GO1305A1, Blue M, New Columbia, PA, USA.) until constant weight (about 48 h) and then ground to pass a 2-mm screen using a ball mill (Thomas Scientific Model: 3375 – E25, Thomas Scientific, Swedesboro, NJ, USA.). The ground samples were stored in 120-mL snap-seal containers for subsequent analyses. Half of the dried sample amounts were kept in the Ruminant Nutrition Laboratory, and the other half were sent for chemical analyses.

Chemical analyses

All forage samples (dried and 2-mm ground) were sent to Cumberland Valley Analytical Services (Hagerstown, MD 21742, USA) for chemical analyses. DM was performed in two steps: the first step was according to Goering and Van Soest (1970), and during the second step, oven temperature increased to 105°C, according to National Forage Testing Association; ash was determined according to AOAC (2002, method 942·05); crude protein (CP) and non-sequential acid detergent fibre (ADF) analyses were performed according to AOAC (2002; methods 2001·11 and 973·18) respectively; neutral detergent fibre (NDF) analysis was determined according to Van Soest *et al.* (1991); ether extract (EE) was determined by AOAC (2002; method 920·39); and lignin (LIG) analysis was performed according to Goering and Van Soest (1970) using 72% sulphuric acid, with modifications (Cumberland Valley Analytical Services Inc; http://www.foragelab.com/resources/labProcedures.cfm).

In vitro gas production measurements

The *in vitro* anaerobic fermentation and gas production of the whole forage were assessed in a fermentation chamber as described by Tedeschi *et al.* (2009). Briefly, the fermentation chamber has twenty-two sensors divided into two sets, 1–11 and 12–22. In each set, a blank bottle (only media + rumen fluid) and a bottle were used in each run, with alfalfa (*Medicago sativa* L.) hay, media and rumen fluid were included as laboratory controls to set fermentation standards. Therefore, twenty-two bottles were used in each run, from which two were blanks, two had alfalfa hay, and eighteen were used to incubate the forage samples. The blanks were used to correct for atmospheric pressure variation and the gas produced by the fermentation of substrates contained in the rumen fluid and the media because Tedeschi *et al.* (2008a) reported that the adjustment for gas production of the blanks have an impact on the fermentation curve. Feed samples (200 mg of 2-mm ground samples) were transferred to a Wheaton bottle (125 mL), which contained a small Teflon-covered stir bar inside to simulate ruminal movements, and wetted with 2 mL of boiled distilled water to avoid sample dispersion, and media was added under anaerobic conditions.

The *in vitro* medium used was the phosphate bicarbonate medium and reducing solution of Goering and

Van Soest (1970). Media and bottles were continuously ventilated with CO_2 to avoid contamination with O_2, and pH was set between 6·8 and 6·9. Saturation was controlled by the colour change of resazurin indicator from purple (rich in O_2) to pink/colourless (lack of O_2). Bottles were filled with 14 mL of medium, closed with butyl rubber stoppers lightly greased, and crimp-sealed with aluminium caps. Strict anaerobic technique was employed in all transfers (Hungate, 1950; Bryant, 1972). The ruminal fluid inoculum was obtained from a non-lactating, rumen-cannulated cow that had free access to medium-quality mixed forages (mostly warm-season grasses). The ruminal fluid used was a mixture of rumen fluid from the dorsal and ventral portions of the rumen. The ruminal fluid was collected approximately at the same time of the day to minimize variation related to circadian feed intake. The collected ruminal fluid was filtered through four layers of cheesecloth and glass wool. A needle was inserted in each bottle, through the rubber stopper, to capture the gas pressure inside the bottle. A pressure sensor was attached to the needle, and the pressure was recorded into a software package (PicoLog, PicoTech, UK) as described by Tedeschi *et al.* (2008b). When the fermentation chamber temperature reached 39°C, 4 mL of the ruminal fluid was added into each bottle. After adding ruminal fluid, the fermentation chamber was closed. When the temperature inside the fermentation chamber reached 39°C, bottles were vented by puncturing the rubber stopper with a needle for 5 s to allow each bottle to start with the same pressure. The fermentation chamber was closed, and when the fermentation reached 39°C, data recording was initiated. Temperature inside the chamber was maintained at 39°C during the fermentation period (48 h). Gas pressure was automatically recorded every 5 min using a computerized system similar to that described by Pell and Schofield (1993).

After 48 h of fermentation (2880 data points were taken by the computerized system, every 5 min), the anaerobic fermentation was stopped, bottles were depressurized, and pH was measured using a digital pH meter. In order to determine the digestible NDF (dNDF), neutral detergent solution (40 mL) (Van Soest *et al.*, 1991) was added to each bottle. Bottles were crimp-sealed, cooked in an autoclave for 60 min at 105°C, filtered by a gravimetric method using a Whatman 54 filter paper using a vacuum system, and dried in oven for 72 h at 60°C. After this period, filters were weighed to estimate undegraded NDF, and forage digestibility was computed by difference.

Statistical analyses

The pressure data measured in each bottle were converted to volume using individual adjustments for each set of bottles and sensors and standardized to 100 mg of sample. The volume of each forage sample was adjusted for the pressure of the blank bottles (average of two bottles). The adjusted volume data were fitted to non-linear models using the Gas Production Fitting System v. 3.2 (GasFit, http://nutritionmodels.tamu.edu) (Tedeschi *et al.*, 2008b) to obtain the kinetic parameters. The following parameters were analysed: (i) asymptote (maximum gas production), mL; (ii) fractional rate of gas production, h^{-1}; and (iii) lag time, h. Preliminary analysis indicated that the two-pool logistic model (Equation 1) had the best fit; therefore, it was selected for further analysis.

$$\text{Gas volume} = a/(1+\exp(2+4\times b\times(c-t))) + d/(1+\exp(2+4\times e\times(c-t))) \quad (1)$$

where a and d are the asymptote of the fast and slow substrate pools, mL; b and e are the fractional degradation rates of the fast and slow substrate pools, h^{-1}; c is the lag time, h^{-1}; and t is time, h^{-1}.

Comparison of equations was made using the Model Evaluation System v. 3.1.4 (MES; http://nutritionmodels.tamu.edu) as described by Tedeschi (2006). Briefly, the mean square error of prediction, the concordance correlation coefficient, and linear regression analysis were used.

Calculation of total digestible nutrients

Forage TDN was used to assess the energy value of the forage. According to Weiss *et al.* (1992), standard analysis alone cannot be used to determine feed energy value. Moran (2005) reported three methods to predict feed digestibility, TDN, and metabolizable energy (ME). Digestibility is not a direct way to measure energy, but it is related to feed nutritive value. The ME is measured as calories or joules per kilogram of DM, and TDN is the sum of the percentages of CP, crude fibre (CF), EE, and nitrogen-free extract that are digested in the gastrointestinal tract of the animal (Weiss *et al.*, 1992).

Weiss *et al.* (1992) proposed a theoretical equation to calculate TDN using concentrations of NDF, LIG, CP, ash, fatty acids or EE, and acid- and neutral detergent-insoluble crude protein (NDICP respectively). The equation has digestion coefficients for CP, lipids, and non-fibre carbohydrate, and it computes digestibility of NDF based on the ratio of LIG to NDF. The metabolic faecal TDN is subtracted to compute apparent TDN. The original equation proposed by Weiss *et al.* (1992) was modified to be used within the Cornell Net Carbohydrate and Protein System (CNCPS; Fox *et al.*, 2004) as the level 1 solution for energy supply. Equation 2 has the form used by the level 1 solution of the CNCPS model.

$$\text{Apparent TDN} = 0{\cdot}98 \times (100 - \text{NDF}_\text{N} - \text{CP - EE} - \textit{ASH}) + \text{k}_\text{dCP} \times \text{CP} + 2{\cdot}25 \times (\text{EE} - 1) + 0{\cdot}75(\text{NDF}_\text{N} - \text{LIG}) \times [1 - (\text{LIG}/\text{NDF}_\text{N})^{0{\cdot}667}] - 7 \quad (2)$$

where NDF_N is the NDF adjusted for nitrogen (NDF – NDF insoluble N), % DM; CP is crude protein, % DM; EE is ether extract, % DM; k_{dCP} is the CP digestibility, %; and LIG is lignin, % DM.

One weakness of Equation 2 is that it does not allow for changes in the digestibility of the NDF among feedstuffs. The main reason is that the values computed by Equation 2 are the TDN for animals with dry matter intake (DMI) at maintenance level (TDN_{1x}); that means values are not discounted for the level of intake as discussed by Tedeschi *et al.* (2005). In order to allow for changes in the digestibility of NDF, Tedeschi *et al.* (2009) developed an equation that computes the digestibility of the NDF using the fractional rates of degradation (kd) of fibre and passage (kp), assuming a linear relationship in the dynamics of fermentation and passage in the rumen. The kd of the NDF was assumed to be the fractional rate of degradation of the second pool (parameter *e*) as shown in Equation 1. Therefore, different kp were tested (4, 6, and 8% h^{-1}) and compared with values predicted by Equation 3. In this model, a 20% intestinal digestibility of NDF (IDNDF) was assumed as proposed by Sniffen *et al.* (1992) for available NDF for all forages. The IDNDF is an adjustment for fibre fermentation in the hindgut.

$$\text{Apparent TDN} = 0{\cdot}98 \times (100 - \text{NDF}_\text{N} - \text{CP - EE} - \textit{ASH}) + \text{k}_\text{dCP} \times \text{CP} + 2{\cdot}25 \times (\text{EE} - 1) + (\text{NDF - NDIN}) \times (\text{kd}/(\text{kd} + \text{kp}) + \textit{IDNDF}) - 7 \quad (3)$$

Where NDF_N is the NDF adjusted for nitrogen (NDF – NDF insoluble N), % DM; CP is crude protein, % DM; EE is ether extract, % DM; k_{dCP} is the CP digestibility, %; kd is fractional rate of NDF degradation, h^{-1}; kp is fractional rate of passage, h^{-1}; and IDNDF is the intestinal digestibility of NDF, % DM.

An adjustment for unavailable carbohydrate (CHO-C = 2·4 × lignin) as proposed by Sniffen *et al.* (1992) and evaluated by Traxler *et al.* (1998) was also investigated as shown in Equation 4.

$$\text{Apparent TDN} = 0{\cdot}98 \times (100 - \text{NDF}_\text{N} - \text{CP - EE} - \textit{ASH}) + k_\text{dCP} \times \text{CP} + 2{\cdot}25 \times (\text{EE} - 1) + (\text{NDF - NDIN} - 2{\cdot}4 \times \text{LIG}) \times (\text{kd}/(\text{kd} + \text{kp}) + \text{IDNDF}) - 7 \quad (4)$$

where NDF_N is the NDF adjusted for nitrogen (NDF – NDF insoluble N), % DM; CP is crude protein, % DM; EE is ether extract, % DM; k_{dCP} is the CP digestibility, %; LIG is lignin, % DM; kd is fractional rate of NDF degradation, h^{-1}; kp is fractional rate of passage, h^{-1}; and IDNDF is the intestinal digestibility of NDF, % DM.

Simulations of energy balance of grazing cows

Simulations to predict animal requirements of ME (Mcal d^{-1}) and metabolizable protein (MP) (g d^{-1}) for maintenance, pregnancy, lactation, and growth and supply of ME and MP by the pastures were performed using the Large Ruminant Nutrition System v. 1.0.1 (LRNS; http://nutritionmodels.tamu.edu), which is based on the CNCPS v. 5 as published by Fox *et al.* (2004). The LRNS-predicted ME and MP balances were used to evaluate whether the chemical analysis and TDN values of the forages were sufficient to explain the observed animal performance throughout the years. For each month of the 4 years of forage sampling, simulations were performed using actual data, which included the chemical analyses of the forages and supplement (Table 1), average temperature, average humidity, wind speed (Table 2) and animal information (except for DMI and expected calf birth weight, which were estimated). Cows were fed 1·45 kg per d of a protein supplement (29% CP), except during the months of July and August. The animal information included days pregnant, days since calving, BW, expected calf birth weight (estimated by the LRNS), BCS, actual supplement intake, and predicted forage intake by the LRNS model. The cow average BW for period 1 (May 2006–April 2007; P1) was 553 ± 71 kg, for period 2 (May 2007–April 2008; P2) was 572 ± 43 kg, and period 3 (May 2008–April 2009; P3) was 580 ± 51 kg (Figure 1). All animals were weighed three times (January, July, and September) during each period as per the management procedures of the King Ranch. Thus, in order to estimate cow monthly BW, a quartic polynomial equation was used (y = 1E-09x^4–3E-06x^3 + 0·0018x^2–0·2292x + 489·67, R^2 = 0·788).

Results

In vitro gas production

The two-pool logistic model had the best fit for all forage samples. The results of IVGP are presented in Table 3. There was a lag time in all fermentations. The average lag times in this south Texas experiment were 6·47 ± 0·54, 7·75 ± 0·65, 7·49 ± 2·01 and 5·44 ± 1·46 h for 2006, 2007, 2008 and 2009 respectively. Ratios of gas produced by 1 mg of DM were 0·41 ± 0·11 mL mg^{-1} in 2006, 0·34 ± 0·09 mL mg^{-1} in 2007, 0·34 ± 0·07 in 2008 and 0·26 ± 0·10 in 2009. The volumes of total gas produced by the second pool were 10·95 ± 2·00 mL, 9·10 ± 1·92 mL, 8·60 ± 1·91 mL and 6·51 ± 1·90 mL, respectively, for

Table 1 Chemical analyses of collected forages in south Texas.

Month	DM, %	ADF	NDF	Lignin	EE	Ash	CP*	ADIN	NDIN	SP
2006 (g kg^{-1}) DM										
February	92·6	423	851	60	13	91	52	22	30	–
March	91·7	515	765	95	11	91	52	14	21	324
April	93·1	345	802	88	14	91	75	34	43	–
May	88·8	328	704	74	16	100	121	40	168	–
June*	95·2	568	807	102	11	79	26	12	13	285
July	90·8	380	655	83	17	99	120	33	161	–
August	90·7	380	621	83	14	92	119	42	70	–
September	92·4	399	640	65	12	88	120	39	105	–
October	94·9	415	753	59	13	97	88	13	28	372
November	93·1	442	736	85	09	85	74	36	78	–
December	95·7	455	764	80	10	93	72	15	26	379
2007										
January	94·7	481	803	102	12	60	73	22	28	311
February	93·1	511	774	116	12	102	69	48	97	–
March	93·1	538	740	105	18	20	68	15	25	273
April	93·2	479	722	93	17	34	69	13	22	370
May	93·1	370	690	57	17	51	117	13	33	457
June	91·7	462	726	88	13	43	62	13	23	367
September	93·8	392	676	73	19	126	112	15	35	436
October	92·5	405	711	77	16	98	119	18	39	387
November	93·4	476	754	80	12	91	49	15	39	353
December	94·6	446	744	91	12	81	47	15	36	266
2008										
January	94·4	502	763	87	11	106	53	18	20	223
February	91·7	517	763	101	11	106	62	19	22	282
March	91·0	463	719	101	12	113	100	26	43	220
April	92·4	571	803	115	04	97	44	18	20	234
May	92·0	608	803	113	07	121	42	16	19	272
June	92·6	475	761	102	11	89	87	26	33	309
July	93·3	362	718	60	22	106	112	16	31	382
August	93·5	359	681	63	31	104	129	16	47	388
September	92·7	381	705	64	22	110	104	14	40	320
October	93·3	452	740	79	11	89	73	19	42	361
November	93·5	441	757	72	12	113	77	19	39	302
2009										
January	93·1	474	778	83	11	108	72	17	28	357
February	91·6	483	785	97	10	94	84	20	29	349
March	87·1	506	820	99	10	62	65	21	22	420
April	93·0	511	812	111	07	65	65	22	24	339
May	91·2	543	813	110	09	79	58	20	21	330
June	86·5	414	689	62	21	114	93	16	29	344
August	91·9	495	791	106	09	74	73	22	30	345
Supplement	89·0	152	319	35	39	76	290	13	50	210

DM, dry matter; ADF, acid detergent fibre; NDF, neutral detergent fibre; EE, ether extract; CP, crude protein; ADIN, ADF insoluble nitrogen; NDIN, NDF insoluble nitrogen; SP, soluble protein, % of CP.

*The CP value is less than expected.

2006, 2007, 2008 and 2009. The fractional degradation rates in these forages were 0·034 ± 0·005 h^{-1} in 2006, 0·033 ± 0·005 h^{-1} in 2007, 0·028 ± 0·004 h^{-1} in 2008 and 0·029 ± 0·005 h^{-1} in 2009.

Total digestible nutrients

The TDN values calculated by Equations 2 and 3 and by the LRNS are presented in Tables 4 and 5. Table 6 has

Table 2 Monthly mean temperatures and precipitation at the King Ranch, Kingsville, TX, for the period of forage collection, and long-term average values.

	January	February	March	April	May	June	July	August	September	October	November	December	Year mean
2006													
Temp °C	16·9	18·8	22·5	26	26·7	28·5	29·3	30·2	26·8	23·5	18·6	15·1	23·6
Prec. (mm)	0·0	2·8	7·5	0·0	72·1	152·3	101·4	20·1	197·5	35·5	0·0	64·9	59·4
2007													
Temp °C	11·8	15·4	20·6	21·1	25·3	27·6	27·8	28·4	27·3	23·5	18·9	17·0	22·0
Prec. (mm)	103·0	0·0	49·1	62·0	228·9	71·1	356·9	57·9	110·4	12·0	7·2	0·0	88·2
2008													
Temp °C	13·5	18·9	19·7	23·1	27·8	29·7	28·0	28·5	26·3	22·5	18·8	15·6	22·7
Prec. (mm)	35·8	0·0	0·8	34·8	20·6	35·7	182·6	172	102·4	24·9	0·0	5·8	51·3
2009													
Temp °C	15·4	18·6	19·3	23·7	27·3	29·4	31	30·2	–	–	–	–	24·4
Prec. (mm)	0·0	4·1	8·7	9·4	65·3	19·5	0·0	38·9	–	–	–	–	18·2
Long term average													
Temp °C	13·0	15·0	19·0	22·0	26·0	28·0	29·0	29·0	27·0	23·0	18·0	14·0	21·9
Prec. (mm)	36·6	43·4	31·5	45·7	89·7	102·0	50·0	77·5	101·1	94·5	38·1	27·2	61·44

the adequacy statistics. The average values for TDN_{1x} without adjustment for CHOC (Sniffen *et al.*, 1992) were 55·9 ± 5·1%, 49·35 ± 5·3% and 45·0 ± 5·3%, respectively, assuming fractional passage rates of 4, 6 and 8% h^{-1} (Table 6). The average TDN_{1x} predicted by Weiss *et al.* (1992) was 53·8 ± 3·4 (Table 6). The prediction using a kp of 8% had the greatest precision (r^2 = 0·67), but the least accuracy (Cb = 0·31). When the adjustment for CHOC was performed (Equation 4), the TDN_{1x} values decreased considerably to 42·8 ± 5·7%, 38·1 ± 5·8%, and 35·1 ± 5·7% for kp of 4, 6, and 8% h^{-1}; respectively (Table 6). Pearson correlations among chemical measurements, TDN, and climate variables are presented in Table 7.

Simulations of the ME and MP balances

The ME and MP balances are presented in Figure 2. For P1, the DMI predicted by the LRNS model for grazing Santa Gertrudis beef cows was not sufficient to meet the ME and MP requirements (negative balance) during all months except for April for MP. The average DMI (forage + supplement) predicted by the LRNS model was 1·75 ± 0·25% of the BW. Similarly, for P2, the DMI predicted by the LRNS model was not sufficient to meet the ME and MP requirements during all periods except for May. The MP balance was negative during May, June, September and October. This was very similar to P1. The average DMI (forage + supplement) predicted by the LRNS model was 1·86 ± 0·21% BW. In the same fashion for P3, the DMI predicted by LRNS model was not enough to meet the energy needs during the entire year and MP balance was negative during all months except July, August and September for MP. The average DMI (forage + supplements) predicted by the LRNS model was 1·86 ± 0·21% of the BW for P3. For all three periods, cows calved in March and calves were weaned in October.

Discussion

In vitro gas production

Similar to our findings, Schofield *et al.* (1994) concluded that single-pool models overpredict values for single substrates when different substrate pools were digested separately, and the parameters were deficient in biological meaning. They also concluded that the variation in mixed substrates cannot be replicated by the exponential curve with dual pools (readily and slowly available carbohydrate fractions). Doane *et al.* (1997) concluded that the best model to fit bromegrass (*Bromus inermis* L.) was the one-pool logistic model although the NDF (55·6%) value of these cool-season

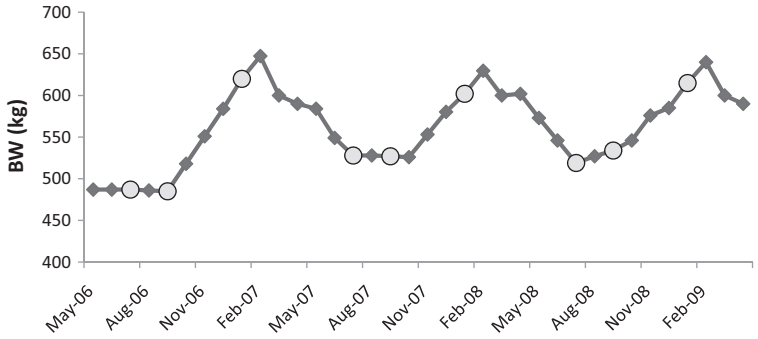

Figure 1 Cow body-weight variation during the collection period. Cows calved in March, and calves were weaned in October. Circles indicate measured body weights.

Table 3 *In vitro* gas fermentation parameters of the whole forage.

Parameters*	January	February	March	April	May	June	July	August	September	October	November	December
2006												
a (mL)	–	5·57	5·29	5·69	5·46	4·27	6·54	6·23	7·37	6·57	7·25	5·84
b (h^{-1})	–	0·164	0·140	0·117	0·122	0·136	0·174	0·149	0·135	0·150	0·155	0·118
c (h)	–	6·87	6·94	6·48	6·11	7·26	6·69	5·71	6·62	6·87	6·05	5·54
d (mL)	–	8·41	10·58	12·14	9·86	8·84	10·65	11·86	14·96	13·26	11·09	8·88
e (h^{-1})	–	0·045	0·032	0·030	0·029	0·036	0·039	0·031	0·032	0·032	0·039	0·033
2007												
a (mL)	3·82	4·25	4·59	4·10	5·32	–	–	4·77	5·19	5·62	4·34	5·65
b (h^{-1})	0·161	0·171	0·179	0·181	0·170	–	–	0·176	0·131	0·099	0·117	0·086
c (h)	8·35	8·38	7·83	7·99	7·25	–	–	8·00	8·26	7·13	7·99	6·33
d (mL)	7·03	9·41	10·18	10·60	11·22	–	–	11·53	9·75	8·51	6·46	6·34
e (h^{-1})	0·040	0·036	0·037	0·036	0·040	–	–	0·034	0·030	0·025	0·032	0·023
2008												
a (mL)	4·89	4·54	4·89	3·70	3·08	4·63	4·59	6·77	4·81	3·18	4·33	–
b (h^{-1})	0·10	0·11	0·10	0·11	0·11	0·14	0·14	0·13	0·13	0·10	0·12	–
c (h)	6·08	5·85	5·66	6·59	9·03	8·03	10·87	8·85	10·27	4·94	6·21	–
d (mL)	9·15	6·55	7·54	7·12	6·32	10·65	9·35	10·67	10·26	6·07	10·96	–
e (h^{-1})	0·026	0·022	0·030	0·027	0·023	0·032	0·031	0·032	0·030	0·026	0·026	–
2009												
a (mL)	6·109	3·87	4·75	5·53	4·75	6·90	–	4·14	–	–	–	–
b (h^{-1})	0·08	0·131	0·087	0·049	0·094	0·132	–	0·109	–	–	–	–
c (h)	3·73	7·69	4·96	3·58	5·83	6·22	–	6·11	–	–	–	–
d (mL)	7·047	7·29	6·06	6·18	4·77	10·01	–	4·23	–	–	–	–
e (h^{-1})	0·028	0·029	0·021	0·032	0·024	0·036	–	0·031	–	–	–	–

*a = total gas production of the first pool (rapidly fermented), mL (1st pool), b = fractional rate of fermentation of the first pool, %/hr, c = Lag time, h, d = total gas production of the second pool (slowly fermented), mL, e = fractional rate of fermentation of the second pool, %/hr.

grass was lower than that of the warm-season forage values (73·9%) from south Texas.

Similar results for lag time were found by Schofield and Pell (1995) working with a cool-season perennial grass, timothy (*Phleum pratense* L.), and a warm-season perennial guineagrass (*Panicum maximum* Jacq.). Schofield and Pell (1995) reported a lag time for timothy and guineagrass of 6·58, and 6·93 h respectively. Miller and Hobbs (1994) suggested the delay in the fermentation might be because IVGP uses dry forage and the forage was not accessible to microbes until they became hydrated. Their results were similar to Schofield and Pell (1995) in which they did not hydrate the samples.

Pell and Schofield (1993) working with alfalfa, bromegrass, timothy, and stargrass (*Cynodon nlemfuensis*

Table 4 Predicted TDN for 2006 and 2007 using two theoretical equations and the Large Ruminant Nutrition System using predicted DMI or assuming DMI as 2·6% of BW for two levels of solutions.

Items	January	February	March	April	May	June	July	August	September	October	November	December
2006												
Weiss *et al.* (1992)	–	53·3	51·4	50·5	56·2	50·9	56·9	55·2	57·9	55·8	55·3	55·1
TDN1x, no adjustment for CHOC												
kp = 4 h^{-1}	–	58·9	55·1	52·1	57·8	57·0	63·9	58·6	60·2	55·6	61·6	57·6
kp = 6 h^{-1}	–	50·6	47·9	44·8	52·8	49·2	59·0	53·4	55·0	48·5	55·1	50·4
kp = 8 h^{-1}	–	45·0	43·2	40·2	49·6	44·0	55·7	50·0	51·6	44·0	50·7	45·8
TDN1x, adjusted for CHOC												
kp = 4 h^{-1}	–	48·4	40·3	38·8	46·9	40·5	50·1	46·0	50·1	46·4	47·5	45·1
kp = 6 h^{-1}	–	41·6	35·3	33·5	43·5	35·1	47·2	42·6	46·4	40·7	43·0	39·8
kp = 8 h^{-1}	–	37·0	32·1	30·2	41·4	31·6	45·2	40·5	44·0	37·1	40·0	36·3
LRNS, using predicted DMI												
Level 1	–	47·0	44·0	47·0	52·0	47·0	54·0	55·0	56·0	52·0	50·0	49·0
Level 2	–	42·0	39·0	42·0	47·0	39·0	49·0	47·0	53·0	52·0	42·0	46·0
LRNS, using DMI = 2·6% BW												
Level 1	–	47·0	44·0	46·0	51·0	45·0	53·0	54·0	54·0	51·0	49·0	48·0
Level 2	–	44·0	37·0	38·0	44·0	34·0	47·0	46·0	51·0	51·0	41·0	45·0
2007												
Weiss *et al.* (1992)	52·3	49·6	58·9	59·1	62·7	58·2	–	–	53·8	54·9	53·9	53·9
TDN1x, no adjustment for CHOC												
kp = 4 h^{-1}	60·8	58·0	66·2	64·7	66·2	62·3	–	–	54·7	52·8	55·9	51·6
kp = 6 h^{-1}	53·0	51·3	59·2	57·8	59·6	55·4	–	–	48·6	46·7	49·0	45·3
kp = 8 h^{-1}	47·9	47·0	54·5	53·3	55·2	50·9	–	–	44·8	43·0	44·5	41·5
TDN1x, adjusted for CHOC												
kp = 4 h^{-1}	43·6	39·3	49·2	49·8	56·6	48·4	–	–	43·7	42·1	43·5	39·3
kp = 6 h^{-1}	38·3	35·4	44·6	45·1	51·4	43·6	–	–	39·3	37·7	38·5	35·0
kp = 8 h^{-1}	34·8	32·8	41·6	42·0	48·0	40·5	–	–	36·5	35·0	35·2	32·4
LRNS, using predicted DMI												
Level 1	48·0	45·0	55·0	56·0	59·0	54·0	–	–	52·0	52·0	49·0	49·0
Level 2	45·0	39·0	49·0	51·0	58·0	53·0	–	–	53·0	51·0	46·0	46·0
LRNS, using DMI = 2·6% BW												
Level 1	47·0	43·0	54·0	54·0	58·0	53·0	–	–	50·0	51·0	49·0	48·0
Level 2	43·0	37·0	48·0	49·0	56·0	50·0	–	–	50·0	50·0	44·0	43·0

TDN, total digestible nutrients.

Harl.) reported a relationship of 0·37 mL of gas produced by 1 mg of DM. Similar results were found by Schofield and Pell (1995) working with timothy, alfalfa, red clover (*Trifolium pratense* L.) and guineagrass (0·39 mL mg^{-1}), while we found a range from 0·26 to 0·41 mL of gas produced by 1 mg of DM. This difference can be explained by the different digestibility levels in the warm-season grasses (33–38%) compared with the levels for timothy (62%) and guineagrass of (58%) reported by Schofield and Pell (1995).

Schofield and Pell (1995) reported total gas production of 15·9 and 16·19 mL 100 mg^{-1} of DM for timothy and guineagrass respectively. The digestibility and the quality of the warm-season grasses from this south Texas experiment were less comparable to those grasses, which can explain the lower gas production from this work. Although the digestibilities of their forages were superior, similar results were found by Schofield and Pell (1995) for timothy degradation rate, 0·032 h^{-1} and for guineagrass, 0·033 h^{-1}.

As mentioned above, the ruminal fluid inoculum was obtained from one cow for all fermentations because preliminary data analysis conducted in our Ruminant Nutrition Laboratory suggested no impact of different ruminal fluid donors (animals) on the pattern of gas production fermentation (Pavan Neto *et al.*, 2007). Based on the review performed by Rymer *et al.* (2005), it is clear that the donor's diet is the main factor that may affect IVGP results. Even different animal species have been reported to yield similar IVGP pattern (Rymer *et al.*, 2005). Vanzant *et al.* (1998) showed that in some *in situ* studies, animals can be responsible for as much as 60% of the variation, but for other studies, animals accounted for <10% of the

Table 5 Predicted TDN for 2008 and 2009 using two theoretical equations and the Large Ruminant Nutrition System using predicted DMI or assuming DMI as 2·6% of BW for two levels of solutions.

Items	January	February	March	April	May	June	July	August	September	October	November	December
2008												
Weiss *et al.* (1992)	50·6	48·9	49·3	48·7	47·2	50·1	56·5	58·8	56·4	54·1	52·2	–
TDN1x, no adjustment for CHOC												
kp = 4 h^{-1}	49·6	46·7	52·9	51·1	46·0	54·5	56·1	59·6	56·1	52·7	49·7	–
kp = 6 h^{-1}	42·8	40·2	46·5	43·8	39·1	47·5	49·5	53·5	49·8	46·3	43·1	–
kp = 8 h^{-1}	38·5	36·3	42·4	39·2	34·9	43·0	45·3	49·6	45·7	42·3	39·0	–
TDN1x, adjusted for CHOC												
kp = 4 h^{-1}	37·1	33·2	37·8	34·3	30·8	38·7	46·9	49·9	46·5	41·4	39·4	–
kp = 6 h^{-1}	32·2	28·8	33·7	29·6	26·3	34·1	41·7	45·2	41·6	36·8	34·4	–
kp = 8 h^{-1}	29·2	26·2	31·0	26·7	23·5	31·1	38·4	42·2	38·5	33·9	31·3	–
LRNS, using predicted DMI												
Level 1	47·0	46·0	47·0	42·0	41·0	44·0	54·0	53·0	50·0	48·0	46·0	–
Level 2	46·0	45·0	41·0	40·0	37·0	40·0	52·0	52·0	45·0	42·0	40·0	–
LRNS, using DMI = 2·6% BW												
Level 1	46·0	45·0	46·0	41·0	40·0	43·0	53·0	52·0	49·0	47·0	45·0	–
Level 2	43·0	43·0	39·0	37·0	32·0	37·0	49·0	50·0	43·0	40·0	38·0	–
2009												
Weiss *et al.* (1992)	50·7	51·5	53·9	52·1	51·2	56·0	–	52·7	–	–	–	–
TDN1x, no adjustment for CHOC												
kp = 4 h^{-1}	50·1	53·6	49·4	57·2	50·5	58·3	–	56·4	–	–	–	–
kp = 6 h^{-1}	43·1	46·4	42·6	49·6	43·4	51·8	–	49·1	–	–	–	–
kp = 8 h^{-1}	38·7	41·9	38·5	44·7	39·1	47·6	–	44·5	–	–	–	–
TDN1x, adjusted for CHOC												
kp = 4 h^{-1}	38·0	39·1	36·5	40·0	35·3	48·3	–	40·3	–	–	–	–
kp = 6 h^{-1}	32·9	34·1	31·7	34·9	30·6	43·3	–	35·5	–	–	–	–
kp = 8 h^{-1}	29·6	31·0	28·8	31·7	27·7	40·0	–	32·4	–	–	–	–
LRNS, using predicted DMI												
Level 1	44·0	43·0	45·0	43·0	44·0	51·0	–	44·0	–	–	–	–
Level 2	44·0	42·0	43·0	41·0	35·0	46·0	–	36·0	–	–	–	–
LRNS, using DMI = 2·6% BW												
Level 1	43·0	42·0	44·0	42·0	43·0	50·0	–	43·0	–	–	–	–
Level 2	42·0	39·0	40·0	37·0	30·0	43·0	–	34·0	–	–	–	–

TDN, total digestible nutrients.

variation. These facts suggest that donor's diet may be the most important factor affecting IGVP results.

Total digestible nutrients

Allen and Mertens (1988) suggested that inside the rumen, there is a selection of particle size for passage (and digestion) and it cannot be measured on *in vitro* studies. Further discussion of the mathematics of fractional passage and digestion rates were provided by Vieira *et al.* (2008a,b). In their work, the fractional passage rate can be modelled using the gamma distribution for the intrinsic transformations that a particle has to undergo in the rumen before it can escape. Furthermore, during filtration most of the microorganisms that degrade fibre stay attached to the solid part of the rumen material (Meyer and Mackie, 1986) and they will appear in the undigested portion of the NDF.

Despite these restrictions, the *in vitro* DM digestibility estimate of the IVGP technique is highly correlated with that predicted by *in vivo* methods (Marten and Barnes, 1980). Van Soest (1994), however, reported that even though systems of chemical analyses are fast and accurate, they do not reflect the biological and nutritional reality that can be reached with *in vitro* systems.

Table 6 illustrates the comparison between different methods in predicting TDN. When compared to the predictions by Weiss *et al.* (1992), the analysis of model adequacy indicated that TDN_{1x} predicted by Equation 3 assuming a kp of 4% h^{-1} had a high accuracy (Cb) of 0·82 and mean bias of −2·19% TDN_{1x}, even though the precision was the least (r^2 = 0·59), points were scattered. The prediction using a kp of 8% h^{-1} had the

Table 6 Model adequacy statistics of the comparison between different methods in predicting TDN.

Comparisons	Mean	s.d.	Median	r^2	MSEP	MB	Cb	CCC	AIC
Weiss *et al.* (1992), Equation 2 with:	53·8	3·4	53·9						
Equation 3 with kp of 4% per h	55·9	5·1	56·1	0·59	15·3	−2·19	0·82	0·63	64·8
Equation 3 with kp of 6% per h	49·3	5·2	49·1	0·64	30·4	4·51	0·61	0·49	59·6
Equation 3 with kp of 8% per h	45·0	5·3	44·5	0·67	87·0	8·79	0·31	0·25	56·6
LRNS level 2 and predicted DMI with:	45·0	5·5	45·0						
Equation 4 with kp of 4% per h	42·8	5·7	42·1	0·54	21·0	2·23	0·93	0·68	105·4
Equation 4 with kp of 6% per h	38·1	5·7	37·7	0·55	64·0	6·96	0·56	0·42	104·3
Equation 4 with kp of 8% per h	35·0	5·7	34·8	0·56	115·1	9·98	0·39	0·29	103·9
LRNS level 2 and 2·6% BW as DMI with:	42·7	6·0	43·0						
Equation 4 with kp of 4% per h	42·8	5·7	42·1	0·60	14·9	−0·13	1·00	0·78	106·7
Equation 4 with kp of 6% per h	38·1	5·7	37·7	0·61	35·9	4·60	0·76	0·60	106·3
Equation 4 with kp of 8% per h	35·0	5·7	34·8	0·60	73·0	7·62	0·54	0·42	106·6

s.d., standard deviation; MSEP, mean square error of prediction; MB, mean bias; Cb, accuracy; CCC, concordance correlation coefficient; AIC, Akaike's Information Criteria; LRNS, Large Ruminant Nutrition System model v. 1·0; TDN, total digestible nutrients

Table 7 Pearson correlations among chemical and nutritional measurements and climatic conditions.

Items†	*a*	*b*	*c*	*d*	*e*	Temperature	Rainfall	NDFD
DM	−0·13	0·08	0·21	0·08	0·17	−0·34	0·20	0·03
ADF	−0·58**	−0·20	−0·14	−0·55**	−0·23	−0·28	−0·35*	−0·58**
NDF	−0·49**	−0·31	−0·19	−0·59***	−0·12	−0·41*	−0·44**	−0·56**
Lignin	−0·54**	−0·21	−0·19	−0·54**	−0·25	−0·18	−0·42**	−0·43**
EE	0·34*	0·35*	0·51**	0·42**	0·26	0·33*	0·50**	0·32*
Ash	0·14	−0·33*	0·05	−0·01	−0·34*	0·08	−0·15	0·27
CP	0·49**	0·19	0·18	0·49**	0·13	0·40*	0·46**	0·58**
ADIN	0·29	0·14	−0·19	0·26	0·15	0·05	−0·13	0·52**
NDIN	0·40*	0·24	−0·40	0·35*	0·20	0·19	0·16	0·68***
Soluble CP	0·32	0·18	0·09	0·24	0·23	0·25	0·41	0·21
TDN_4								0·80***
TDN_6								0·84*
TDN_8								0·85*
TDN_{Weiss}								0·52

*$P < 0·05$.

**$P < 0·01$.

***$P < 0·001$.

†*a*, *b*, *c*, *d* and *e* are parameters of the two-pool logistic non-linear function; EE, ether extract, % DM; TDN_4, TDN_6 and TDN_8 are TDN estimated at maintenance level of intake (TDN_{1x}) not adjusted for unavailable carbohydrate, assuming 4, 6 and 8% h^{-1} passage rate, respectively, and TDN_{Weiss} is TDN_{1x} predicted by the Weiss *et al.* (1992) equation.

ADF, acid detergent fibre; CP, crude protein; DM dry matter; NDF, neutral detergent fibre; NDIN, NDF-insoluble nitrogen; TDN, total digestible nutrients.

greatest precision ($r^2 = 0·67$) but the least accuracy (Cb = 0·31). When the adjustment for CHOC was performed (Equation 4), the TDN_{1x} values decreased considerably to 42·8 ± 5·7%, 38·1 ± 5·7% and 35·1 ± 5·7% for kp of 4, 6 and 8% h^{-1} respectively (Table 6). This suggested that the inclusion of CHOC in predicting TDN_{1x} was likely to significantly decrease the predicted performance of the animals even though the fractional rate of fermentation should be associated with the available NDF and not the total NDF.

The average TDN values calculated by the LRNS model using the predicted DMI were 48·8 ± 4·5% and 45·0 ± 5·5%, respectively, for levels of solution 1 (Equation 2) and 2. The level of solution 2 uses the mechanistic rumen submodel and the individual fractional degradation rates of the feed carbohydrate and protein fractions (Fox *et al.*, 2004). Both TDN values predicted by levels 1 and 2 were discounted by the level of intake as discussed by Tedeschi *et al.* (2005). Sprinkle (1996) reported that the DMI necessary to meet

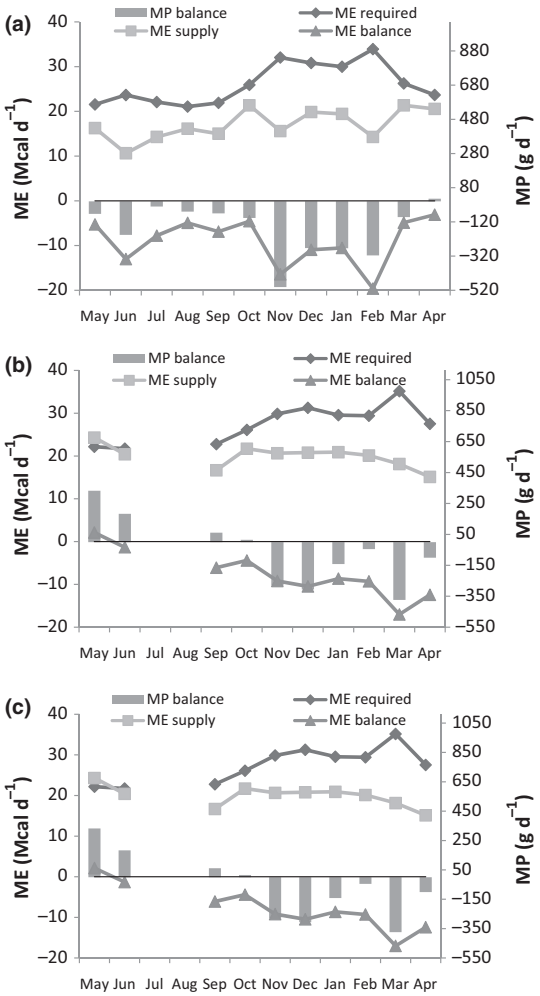

Figure 2 The metabolizable energy and protein balances for May 2006 to April 2007 (a), May 2007 to April 2008 (b) and May 2008 to April 2009 as predicted by the Large Ruminant Nutrition System model.

maintenance requirements in beef cows consuming forage (TDN = 55%) was about 2·6% of BW. When DMI was used as 2·6% of BW, the TDN values for levels 1 and 2 were 47·8 ± 4·5 and 42·7 respectively. The decrease in TDN values was expected because the LRNS accounts for level of intake to predict fractional passage rate; as DMI increases the fractional passage rates also increases, and therefore, the predicted TDN decreases (Fox *et al.*, 2004).

The average DMI reported in the literature (Hatfield *et al.*, 1989; Juarez Lagunes *et al.*, 1999; and Sowell *et al.*, 2003) for free-ranging beef cows was 2·6% of BW, suggesting the LRNS (i.e. CNCPS) may have underestimated the DMI intake for grazing cows in south Texas. Therefore, the values obtained by the LRNS (either using predicted DMI or the 2·6% BW to predict DMI) were likely to yield more realistic numbers because the model simultaneously accounts for CHOC and discounts for level of intake. The values predicted by Equations 2–4 were the TDN_{1x}; thus, they have to be discounted as suggested by Tedeschi *et al.* (2005) for more realistic predictions of animal performance when using a model that does not account for these factors in predicting nutritive values of feeds. When TDN was compared and predicted by the level 2 of solution of the LRNS pool (fibre) was used to compute TDN. The correlation between TDN_4, TDN_6 and TDN_8 with TDN_{Weiss} (Equation 2) were high (0·62, 0·66, and 0·68 respectively) and slightly increased with kp values.

According to Getachew *et al.* (2005), IVGP data can be used to predict the energy value of forages. Menke *et al.* (1979) reported a high correlation (r = 0·98) for predicting ME values of feedstuffs using IVGP with measured ME ranging from 7·7 to 13·2 MJ ME kg^{-1} DM, and an average of 11·17 ± 1·08 with an equation s.d. of 0·25 MJ. Other values in the literature indicated a 15% variation in ME values predicted by IVGP techniques compared with those predicted using other *in vivo* techniques (Krishanmoorthy *et al.*, 1995). These authors concluded that IVGP can be used to predict energy values. Iantcheva *et al.* (1999) also reported that IVGP can be used to estimate energy values of forages and reported regression equations for alfalfa (r = 0·86–0·93) and grass hay (r = 0·83–0·91).

Based on our evaluations of the south Texas warm-season forages, a kp of 4% h^{-1} may reflect the typical passage rate in beef cows grazing low-to-moderate forage quality. The average TDN for kp of 4% h^{-1} was 55·9% (Table 6). The NRC (2000) suggested that TDN ranged from 53 to 57% in forages when the passage rate was 4% h^{-1}. The predicted kp by the LRNS model using the level 2 solution averaged 3·66% h^{-1}. This value is in agreement with the assumption of using 4% h^{-1} as the expected kp of these grazing Santa Gertrudis cows. The comparison of TDN_4 and TDN_{Weiss} indicated IGPV may overpredict TDN_{Weiss} (55·9 vs. 53·8 respectively, Table 6).

Relationships of chemical analyses and *in vitro* parameters

Moreira *et al.* (2004) working with stargrass reported positive correlations between leaf NDF and leaf ADF with digestibility (r = 0·73 and 0·46 respectively); however, they found a negative correlation between NDF and ADF with digestibility (r = −0·58 and −0·56 respectively). The negative correlation between digestibility and fibrous parameters might be related to the ratio of

stem to leaf, and it is likely that the forages in this study had a greater stem to leaf ratio. Moore and Jung (2001), Casler and Jung (2006) and Yayneshet *et al.* (2009) all reported a negative correlation between digestibility with LIG and NDF. Those data agree with those of the south Texas warm-season grasses in which there was a negative correlation ($r = -0{\cdot}43$) between LIG and *in vitro* DM digestibility.

The positive correlation between CP and digestibility ($r = 0{\cdot}58$) agrees with results reported by Getachew *et al.* (2004) and Ammar *et al.* (2004). When compared to Archer and Decker (1977) and Ammar *et al.* (2004), the negative correlation between fibrous parameters of the warm-season grasses kleberg grass and coastal bermudagrass; however, correlations were greater. In contrast to Getachew *et al.* (2004), there was a positive correlation between CP and parameter d ($r = 0{\cdot}49$), a small correlation between parameter e and CP ($r = 0{\cdot}13$), and a positive correlation between SP and parameter *d*.

The small negative correlation between LIG and temperature was not expected but is in agreement with Buxton and Redfearn (1997), who reported that lignified tissues provide resistance to support low temperatures and protection against diseases and insects. Ford *et al.* (1979) working with tropical and temperate grasses reported a positive correlation between temperature and LIG. The variance in those results may be explained by the difference in plant maturity. The weak correlation between LIG and parameters *b*, *c* and *e* agrees with Robinson *et al.* (2004); however, a moderate correlation between parameter *a* and *d* with LIG ($r = -0{\cdot}54$) was also found. Although LIG is not bound to cellulose (Jung and Ralph, 1990), the amount of LIG may influence the accessibility of microbes to substrate, and cell wall contents (Mandebvu *et al.*, 1999), and consequently influence the amount of gas produced. The positive correlation between rain and digestibility ($r = 0{\cdot}37$) agrees with Pitman and Holt (1982), who examined warm-season perennial grasses Kleingrass 75 (*Panicum coloratum* L.), Kleingrass 75-25 (*Panicum coloratum* L.), green sprangletop [*Leptochloa dubia* (H.B.K) Nees], and plains bristlegrass (*Setaria macrostachya* H.B.K.).

Simulations of the ME and MP balances

The accurate predictions of DMI and animal response under grazing systems with tropical grasses require adequate measurements of NDF, LIG, CP, SP and digestion rates for fibre and protein (Juarez Lagunes *et al.*, 1999). Allison (1985) reported that if animals on rangeland could consume enough forage, they could meet their requirements, although DMI is affected by animal and plant physical factors and by plant–animal interactions. Sprinkle (1996) suggested that if not enough forage is available, no supplementation programme will be helpful to achieve sufficient nutrient requirements. Based on previous discussion, ME and MP balances were simulated using a DMI of 2·6% (forage + supplement) of BW and assumed that cows were consuming on average 0·29, 0·26 and 0·22% of BW, respectively, of supplement for P1, P2 and P3. The DMI of forage was computed as the difference between total DMI and the supplement intake, and no forage substitution or increase in forage intake was assumed.

The ME and MP balances were negative for most of the simulated period. Period 3 was during the intense dry period in south Texas, and this may have influenced the nutritional requirements, forage availability, and consumption. Range animals can have their nutritional requirements altered by grazing activity, travel, and environmental stress (Allison, 1985). Nonetheless, the negative simulated ME and MP balances were not consistent with the change in BW of the cows (Figure 1) during the three periods. Figure 1 supports the hypothesis that cows had an overall positive MP and ME balance throughout the reproductive cycle because the average BW of the cows increased during these periods.

Winter- or spring-calving cows are usually in negative energy balance before calving owing to the low intake of nutrients. May and June are likely to be when peak milk production occurred for these Santa Gertrudis cows. Offering the necessary quantity and the proper supplementation to beef cows can improve the utilization of low-quality forage (Ovenell *et al.*, 1991). The amount of nutrients necessary for positive energy balance was high, and animals would have to consume large amounts of forage because of its low quality. However, in this case, the physical capacity of the rumen would be the first limiting factor. The poor quality of the forage and increase in MP and ME requirements during these months likely contributed to the negative protein and energy balance. According to Baumann *et al.* (2004), low-quality forage usually does not supply either energy or protein requirements to beef cows during early lactation in order to maintain BW and body condition score.

Nutrition models based on the CNCPS technology are sensitive to feed chemical analysis and fermentation kinetics, and accurate predictions of forage DMI and composition of the feeds are needed to adequately predict energy balance and supplementation strategies of grazing beef cows. Our evaluation indicated that cows were able to maintain reproductive status (Figure 1), despite model predictions for ME and MP balances being negative for most of the time. This suggests that further work might be needed to improve the model predictions, or concomitantly, it is possible the forage sampling technique used was not adequate

for this environment and/or the forage samples were not representative of the forage consumed by the animals. The review by Holechek *et al.* (1982) suggested that even though hand plucking forage samples based on visual appraisal of animal's consumption is adequate, direct observation may not be adequate or practical on large brush-infested pastures. In addition, maintenance requirement of these animals might be lower than predicted by the model.

Implications

The IVGP is a non-invasive method that requires smaller amounts of sample and less labour. It can be used to estimate degradation rates of feedstuffs, and when combined with chemical analysis, it can assist producers to improve animal productivity and make grazing management decisions. In addition, IVGP can estimate digestibility, fermentation kinetics and volatile fat acid profiles concurrently. Different forages, animal *per se*, and level of production have an effect on the passage rate, and different passage rate have to be used to estimate TDN value. The variation in the forage fermentation and, consequently, TDN values may affect animal performance (pregnancy and conception rates) that require close monitoring of forage quality and supplementation strategies to maintain level of production and profitability. Different methods to assess the quality of the forage might be needed under the conditions of this study because of the lack of agreement between observed and model predicted cow's performance.

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